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Application of *in situ* hybridization procedure on tissue sections to identification of molds causing invasive fungal infections

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Early, rapid, and accurate diagnosis of invasive fungal infections (IFIs) is essential for appropriate antifungal therapy, whereas the morphological similarities of many molds in tissue make their specific diagnosis difficult. Hence it is required to have a rapid and accurate method of diagnosis of IFIs in surgical pathology specimens. This study was carried out in order to find the usefulness of *in situ* hybridization (ISH) to identify various kinds of molds observed in tissue section and/or cytological specimen from the patients with invasive fungal infections. To establish the precise procedure for ISH in formalin-fixed and paraffin-embedded sections, various methods of pretreatment were tested. An excellent outcome was found in staining intensity and specificity on molds observed in the tissue sections, when specimens were treated with both heat and proteinase K, and were heating solutions were adjusted to higher pH value. In addition, it was examined that intensity and specificity of two each DNA and peptide nucleic acid (PNA) probes, using experimentally infected lung of mice and lung of autopsies with invasive mold infection confirmed by culture. As the result, DNA probes targeting the alkaline proteinase (*ALP*) gene and retrotransposon *Afut*-1 gene of *Aspergillus fumigatus* showed specificity for the *Aspergillus* species and *Aspergillus fumigatus*, respectively. PNA probes for *C. albicans* and *Fusarium* species also showed satisfactory specificity. We wish to emphasize that ISH is significant to be a valuable tool to identify medically important molds on formalin-fixed and paraffin-embedded sections or cytological preparations. The application of PNA probe is especially attractive as a choice for clinical diagnosis due to decreased test turnaround.

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In situ immunodiagnosis of mycoses

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As a consequence of the difficulties in even suspecting the presence of deep-seated mycoses clinically, many cases are not diagnosed until tissue specimens are examined histologically. However, it must be appreciated that although special histochemical stains for fungi, like e.g. periodic acid-Schiff (PAS) and methenamine-silver techniques (Grocott), are useful for revealing the presence of fungal elements in tissues, they seldom permit the exact fungal genus involved to be identified. Histologically, distinctive morphological details may provide a tentative identification, but the appearance of fungi in sections is affected by steric orientation, age of the fungus, etc. Moreover, the elements of some of the most emerging fungal pathogens, i.e. species of Aspergillus, Fusarium, and Scedosporium cannot be differentiated in tissues due to morphological similarities. Also the presence of sparse and/or atypical fungal elements will hamper a clear-cut diagnosis and may result in confusion of e.g. aspergillosis, fusariosis, and scedosporidiosis with zygomycosis and candidosis, respectively.

As the therapy of deep-seated mycoses is becoming more and more specific and is directed by the fungal genus or even the species involved, there is an increasing demand for specific and reliable in situ diagnoses.

Highly sensitive and specific, indirect immunohistochemical techniques have been developed for the identification of the most prominent causes of mycoses. Moreover, as a range of different forms of fungal elements frequently is disclosed both in isolated lesions and/or in different organs of individuals, dual immunostaining techniques are often mandatory for obtaining a reliable and discriminative diagnosis.

An important limitation of the widespread application of immunohistochemical techniques for the diagnosis of deep-seated mycoses is due to the fact that sensitive and specific reagents are obtained through multiple heterogeneously absorbed polyclonal antibodies, which are not commercially available. However, in recent years more specific monoclonal antibodies have been commercialized though companies offering immunodiagnostic reagents.